

## **CHARACTERIZATION AND FRACTIONATION IN AQUEOUS TWO-PHASE SYSTEMS OF SITE-SPECIFIC PEGYLATED ANTIBODIES: TARGETING STEM CELL SEPARATION**

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**Key Words:** Antibody PEGylation, aqueous two-phase systems, affinity, stem cells.

Affinity aqueous-two phase systems (ATPS) are constructed by complementing ATPS with the use of antibodies. This novel methodology represents an attractive alternative for the recovery of stem cells that exhibits several advantages including: biocompatibility, economical attractiveness, scalability, and low processing time. Moreover, the PEGylation of the added antibody with a freely activated site exploiting a site-specific PEGylation via biotin-streptavidin conjugation could confer distinctive characteristics that can be employed in the separation of CD133<sup>+</sup> stem cells from human umbilical cord blood samples. Currently, we are performing the site-specific PEGylation of CD133-Biotin antibody with the next six activated biotinylated PEGs: thiol, maleimide, carboxyl, amine, methoxy poly(ethylene glycol), and succinimidyl ester, in order to characterize them and compare their partition behavior utilizing electrophoresis with silver staining and I<sub>2</sub>-BaCl<sub>2</sub> in the following three polymer-polymer aqueous two-phase systems (ATPS): ficoll 400,000-dextran 70,000, PEG 8,000-dextran 500,000, and the novel UCON-dextran 75,000. We hypothesize that the freely activated site may influence the partitioning profile of the PEGylated CD133-Biotin antibody into opposing phases of the contaminants, thus providing a suitable affinity ATPS for the recovery of CD133<sup>+</sup> stem cells.